

WHAT IS CLAIMED IS:

1. Apparatus for detecting fluorescently marked regions on a substrate, said apparatus comprising:
 - 5 a light source;
 - an optical train for directing a light from said light source at said substrate;
 - means for focusing said light on a surface of said substrate;
 - 10 means for detecting a fluorescent emissions from said fluorescently marked regions in response to said light;
 - means for translating said substrate from a first position to a second position; and
 - means for storing a set of values representing an intensity of said fluoresced light, said intensity being a function of the location of said substrate.
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2. Apparatus as recited in claim 1 further comprising a video display means for displaying said values representing the intensity of said fluoresced light as a function of location of said substrate.
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3. Apparatus as recited in claim 1 wherein said optical train comprises:
 - 25 a spatial filter comprising of a first and a second lens and a confocal pinhole located between said first and said second lens;
 - a beam splitter cube;
 - a dichroic mirror for passing light having a wavelength of about said fluorescence emissions and reflecting light having a wavelength of about said light;
 - 30 an optical lens; and
 - a microscope objective for directing said light at said substrate.
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4. Apparatus as recited in claim 1 wherein said focusing means comprises:

a photodiode for generating a voltage representing an intensity of said light reflected from said substrate; and

a focusing lens for focusing said reflected light from said optical train at said photodiode;

5 means for moving said substrate relative to a microscope objective until said light detected from said substrate substantially reaches a maximum.

10 5. Apparatus as recited in claim 4 wherein a confocal pinhole is located between said focusing lens and said photodiode.

6. Apparatus as recited in claim 1 wherein said detecting means comprises:

15 a photomultiplier tube; and
a lens for focusing said fluorescent emissions collected by said optical train at said photomultiplier tube.

20 7. Apparatus as recited in claim 6 wherein a confocal pinhole is located between said focusing lens and said photodiode.

25 8. Apparatus as recited in claim 6 wherein said photomultiplier tube is couple to a means for collecting pulses generated by said photomultiplier tube in response to an intensity of said fluorescent emissions, said means for collecting pulses connected to a programmable computer for storing and analyzing said pulses.

30 9. Apparatus as recited in claim 1 wherein said translating and said focusing means comprise a x-y-z translation table, a flow cell mounted on said x-y-z translation table, said flow cell comprising a mounting surface with a cavity therein, said mounting surface comprises
35 means for mounting said substrate thereon and maintaining a sealed relationship with said substrate, said cavity comprises an inlet and an outlet, and said inlet connected to a pump for

transferring materials into said cavity and out through said outlet.

10. An apparatus as recited in claim 9, further
5 comprising means for controlling temperature in said flow cell, said means for controlling temperature including a recirculating bath device for circulating water through channels disposed in said flow cell.

10 11. An apparatus comprising:
a light source;
an optical train for directing light from said light source at a substrate including a surface having fluorescently marked regions;
15 a translation system co-operatively arranged with said optical train and constructed to support and displace said substrate;
an auto-focusing system constructed and arranged to focus said directed light onto said surface;
20 a detector for detecting fluorescent light from said fluorescently marked regions of said surface in response to said light; and
a computer arranged to control operation of said light source, said detector, said translation system and
25 said auto-focusing system to execute auto-focusing by controlling said translation system and bringing into focus corners of said surface; said computer being further arranged to receive data from said detector corresponding to said detected fluorescent light and provide a data file
30 representing an array of photon counts as a function of a pixel position on said surface.

12. The apparatus of claim 11, wherein said computer is further arranged to generate an image file including data indicative of fluorescence intensity level as a function of said substrate pixel position.

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13. The apparatus of claim 11, wherein said detector comprises a confocal detector including a pinhole.

14. The apparatus of claim 11, wherein said detector
10 comprises a photodiode utilized for said auto-focusing and a photomultiplier for detecting said fluorescent light.

15. The apparatus of claim 11, wherein said computer executes said auto-focusing by interpolating focusing values
15 determined for said corners of said surface having a planar shape.

16. The apparatus of claim 11, wherein said computer executes said auto-focusing by bringing into focus all four
20 of said corners of said surface.

17. The apparatus of claim 16, wherein said computer executes said auto-focusing by interpolating focusing values determined for said four corners of said surface having a
25 planar shape.

18. The apparatus of claim 11, wherein said translation system includes an x-y-z- translation stage.

30 19. The apparatus of claim 11, wherein said optical train separates reflected excitation light from said surface of said substrate from fluoresced light from said surface.

20. An apparatus comprising:

a light source constructed to emit excitation light;

5 an optical train for directing said excitation light from said light source at a substrate including a surface having fluorescently marked regions;

a translation system co-operatively arranged with said optical train and constructed to support and displace
10 said substrate;

an auto-focusing system constructed and arranged to focus said excitation light onto said surface;

a detector for detecting fluorescent light from said fluorescently marked regions of said surface in
15 response to said excitation light; and

a computer arranged to receive data from said detector corresponding to said detected fluorescent light of individual pixels of said surface and determine a dynamic range for data scaling; said computer being further arranged
20 to scale said data and provide a data file representing an array of photon counts as a function of a pixel position on said surface.

21. The apparatus of claim 20, wherein said computer
25 is further arranged to scale said data using logarithmic scaling.

22. The apparatus of claim 21, wherein said computer is further arranged to generate an image file including data
30 indicative of fluorescence intensity level as a function of said substrate pixel position.

23. The apparatus of claim 20, wherein said computer is further arranged to scale said data using linear scaling.

24. The apparatus of claim 23, wherein said computer
5 is further arranged to generate an image file including data indicative of fluorescence intensity level as a function of said substrate pixel position.

25. The apparatus of claim 24, wherein said computer
10 is arranged to control operation of said light source, said detector, said translation system and said auto-focusing system to execute auto-focusing by controlling said translation system and bringing into focus corners of said surface

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26. The apparatus of claim 25, wherein said auto-focusing system determines a focal plane of the light passing through said optical train.

20 27. The apparatus of claim 26, wherein said optical train separates reflected excitation light from said surface of the substrate from fluoresced light from said surface.